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The *cysP* Promoter of *Salmonella typhimurium*: Characterization of Two Binding Sites for CysB Protein, Studies of In Vivo Transcription Initiation, and Demonstration of the Anti-Inducer Effects of Thiosulfate

MONIKA M. HRYNIEWICZ† AND NICHOLAS M. KREDICH*

Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

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The *cysPTWA* operons of *Escherichia coli* and *Salmonella typhimurium* encode components of periplasmic transport systems for sulfate and thiosulfate and are regulated as part of the cysteine regulons. In vitro transcription initiation from the *cysP* promoter was shown to require both CysB protein and either *O*-acetyl-L-serine or *N*-acetyl-L-serine, which act as inducers, and was inhibited by the anti-inducer sulfide. Thiosulfate was found to be even more potent than sulfide as an anti-inducer. DNase I protection experiments showed two discrete binding sites for CysB protein in the presence of *N*-acetyl-L-serine. CBS-P1 is located between positions −85 and −41 relative to the major transcription start site, and CBS-P2 is located between positions −19 and +25. Without *N*-acetyl-L-serine, the CysB protein protected the region between positions −63 and −11, which was designated CBS-P3. In gel mobility shift assays, the mobility of CysB protein-*cysP* promoter complexes was increased by *O*-acetyl-L-serine. *N*-Acetyl-L-serine had no effect in gel shift experiments, presumably because its anionic charge results in its rapid removal from the complex during electrophoresis. Comparison of DNA fragments differing with respect to binding site position indicated that complexes with CysB protein contain DNA that is bent somewhere between CBS-P1 and CBS-P2 and that *O*-acetyl-L-serine decreases DNA bending. Binding studies with fragments containing either CBS-P2 alone, CBS-P1 alone, or the entire *cysP* promoter region suggest a model in which the complex of bent DNA observed in the absence of *O*-acetyl-L-serine contains a single CysB protein molecule bound to CBS-P3. At relatively low CysB protein concentrations, *O*-acetyl-L-serine would cause a single CysB protein molecule to bind tightly to CBS-P1, rather than to CBS-P3, thereby decreasing DNA bending and increasing complex electrophoretic mobility. At higher CysB protein concentrations, *O*-acetyl-L-serine would cause a second molecule to bind at CBS-P2, giving a more slowly migrating complex.

Assimilatory sulfate reduction in *Salmonella typhimurium* and *Escherichia coli* commences with the uptake of extracellular sulfate, a process requiring a periplasmic transport system termed the sulfate permease system (7, 8, 43). All but one of the components of the sulfate permease system are encoded by contiguous genes located at 52 min on the *E. coli* map (3, 20) and at 49 min on the *S. typhimurium* map (8, 48), which in *S. typhimurium* were originally designated *cysAa*, *cysAb*, and *cysAc* (31). This genetic region has recently been cloned and sequenced in *E. coli* and found to contain five open reading frames, which, beginning furthest upstream, were designated *cysP*, *cysT*, *cysW*, *cysA*, and *cysM* (13, 50). *cysM* encodes *O*-acetylserine (thiol)-lyase B, which catalyzes the synthesis of L-cysteine from *O*-acetyl-L-serine and sulfide (4, 15) and also the synthesis of *S*-sulfocysteine from *O*-acetyl-L-serine and thiosulfate (35, 36). The deduced amino acid sequences of *E. coli cysT*, *cysW*, and *cysA* suggest they encode the three membrane-bound components that are typical of periplasmic substrate-binding transport systems (2), and these three genes probably correspond to *S. typhimurium cysAa*, *cysAb*, and *cysAc*, respectively. *cysP* has been shown to encode a periplasmic thiosulfate binding protein (13) that is similar to but distinct from the sulfate binding protein from *S. typhimurium* (9, 16, 42, 43). Since mutants lacking sulfate permease are also defective in thiosulfate uptake (7), it now appears that the transport activities of these two anions utilize the same three membrane components specified by *cysT*, *cysW*, and *cysA* but require separate periplasmic binding proteins (13, 50). The gene encoding the sulfate binding protein has not been identified in spite of a systematic search (38).

Sulfate and thiosulfate transport activities vary according to the availability of L-cysteine and reduced sulfide (7) and are presumed to be regulated, together with other activities required for L-cysteine biosynthesis, at the gene level as part of the cysteine regulon (21, 22, 38). The expression of the cysteine regulon is controlled by the CysB protein, which serves as a specific transcription activator for genes of the biosynthetic pathway (18, 21, 34, 39) and as a repressor for its own gene, *cysB* (5, 17, 41). Positive regulation also requires sulfur limitation and an inducer, which can be either *O*-acetyl-L-serine or *N*-acetyl-L-serine (19, 21, 34, 39). At least part of the need for sulfur limitation is due to the fact that sulfide is an anti-inducer of the cysteine regulon (40).

In vitro studies have demonstrated that CysB protein binds to a site immediately preceding the −35 regions of the positively regulated *cysJIH* and *cysK* promoters and that binding is stimulated by acetyl-L-serine, i.e., *O*-acetyl-L-serine or *N*-acetyl-L-serine (34, 40). The combination of CysB protein and acetyl-L-serine markedly stimulates in vitro transcription initiation from both promoters, and this effect is inhibited by the anti-inducer sulfide (32, 39, 40). In

* Corresponding author.
† Permanent address: Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland.

vitro binding of CysB protein to the *cysK* promoter induces DNA bending, which is diminished or eliminated by acetyl-L-serine (34). DNA bending at the *cysK* promoter requires the presence of two adjacent CysB protein binding sites and has not been observed with the *cysJIH* promoter, which has only a single binding site.

The presence in *E. coli* of overlapping start and termination codons between *cysP*, *cysT*, and *cysW*, a small overlap of the coding regions of *cysW* and *cysA* (50), and the polar effect of a *cysP* mutation (13) suggest that these four genes comprise a single operon, which is expressed from the *cysP* promoter. An apparent rho-independent terminator located between *cysA* and *cysM* indicates that the later may be expressed from its own promoter. An in vivo transcription start site for the *E. coli cysP* promoter has been demonstrated with primer extension analysis (13). In this report we identify the in vivo start site for the *S. typhimurium cysP* promoter and characterize in vitro interactions of this promoter with the CysB protein, *O*-acetyl-L-serine, and *N*-acetyl-L-serine. We also show that thiosulfate is a potent anti-inducer of the cysteine regulon.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. *E. coli* NM522 [*hsdΔ5* Δ(*lac-pro*) (F′ *pro*$^+$ *lacI*^{q}*ZΔM15*)] was used as a host for pT7T3 derivatives. *E. coli* EC1250 (F$^-$ *araD139* Δ*lac U169 rpsL thi fla trp6*) (17), wild type *S. typhimurium* LT2, its derivative carrying pRSM16, and *S. typhimurium cysA20* were used as sources of RNA. *cysA20* has a 4-kb deletion in the *cysA* region (14). pRSM16 contains *S. typhimurium cysPTWA* (formerly known as the multicistronic *cysA* region) on a 6.2-kb fragment inserted into the *Bam*HI site of pBR322 (33). A 1.07-kb *Bam*HI-*Eco*RI portion of this insert, containing the *cysP* promoter and 0.73 kb of *cysP* coding region (see below), was subcloned into the *Bam*HI site of pT7T318U to give pMHK1. A 0.31-bp *Hin*dIII-*Hae*II fragment from pMHK1 was made blunt ended at the *Hae*II site and inserted between the *Hin*dIII and *Sma*I sites of pT7T319U to give pMHK7. This insert consisted of approximately 30 bp of polylinker sequence from pT7T318U plus 283 bp from the upstream portion of the *cysP* promoter (see below).

Double-strength YT medium (30) was used for the growth of NM522 and was supplemented with 100 μg of ampicillin and 70 μg of kanamycin per ml for the production of single-stranded phagemid DNA (28). Medium E (51) prepared with an equimolar amount of $MgCl_2$ in place of $MgSO_4$ served as our minimal salts medium and was supplemented with 0.5% glucose and either 0.5 mM L-cystine or 1.0 mM L-djenkolic acid as a sulfur source. L-Tryptophan at 0.2 mM and thiamine at 4 μg/ml were included for growth of EC1250.

Recombinant DNA methods. Our general recombinant DNA methods were those described previously (26). DNA sequencing was performed by the method of Sanger et al. (49) with specific oligodeoxynucleotide primers and single-stranded templates derived from pT7T3 derivatives (28). In some cases, 5′-labeled DNA was also cleaved chemically (27) to generate size markers. Oligodeoxynucleotides were prepared with an automated DNA synthesizer from Applied Biosystems (model 380A). The polymerase chain reaction (PCR) was performed with a reagent kit from Perkin Elmer Cetus. Reaction mixtures contained 2 to 6 ng of template DNA, 100 pmol of each oligodeoxynucleotide primer, and 2.5 U of Taq polymerase in 100 μl of 10 mM Tris-hydrochloride (pH 8.3)–50 mM KCl–1.5 mM $MgCl_2$–0.2 mM of each deoxynucleoside triphosphate–0.01% gelatin and were incubated for 30 cycles at 94°C for 1 min, 55°C for 1.5 min, and 72°C for 0.5 min. Where required, one of the two oligodeoxynucleotides was 5′ labeled with γ-^{32}P-ATP (3,000 Ci/mmol) and T4 polynucleotide kinase (27). The mixture was then extracted with phenol-chloroform-isoamyl alcohol (25:24:1); after precipitation with ethanol, the PCR products were identified by agarose gel electrophoresis and autoradiography. Radiolabeled DNA fragments were usually used without further purification in DNA binding assays but were purified by polyacrylamide gel electrophoresis for DNase I footprinting experiments.

Analysis of in vivo transcripts. Total cellular RNA was prepared by the method of Aiba et al. (1) from bacteria grown on minimal medium containing either L-cystine acid or L-djenkolic as a sulfur source. Primer extension and S1 nuclease protection studies were performed as described previously (6, 39).

In vitro runoff transcription assays. A DNA fragment containing the *S. typhimurium cysP* promoter was generated from pMHK1 by using the PCR. For control studies, a 458-bp *Bgl*II-*Pvu*II fragment containing the phage λ p_L promoter with its start site oriented 325 bp upstream of the *Pvu*II end (44) was obtained from D. Steege. Transcription initiation complexes were formed by incubating various amounts of CysB protein, *O*-acetyl-L-serine or *N*-acetyl-L-serine, and other effectors in 20 μl of 40 mM Tris-hydrochloride (pH 8.0)–0.1 M KCl–10 mM $MgCl_2$–1 mM dithiothreitol–0.1 mM ATP containing approximately 20 ng of template DNA, 1 ng of nuclease-free *E. coli* RNA polymerase (Pharmacia-LKB Biotechnology Inc.), and 2 μg nuclease-free bovine serum albumin (34, 39). After 5 min at 37°C, transcription elongation was begun by adding 2 μl of a solution containing 20 μM α-^{32}P-CTP (200 Ci/mmol), 2 mM each ATP, GTP, and UTP, and 0.5 mg of sodium heparin per ml. After another 5 min at 37°C, the reaction was terminated by the addition of 0.2 ml of 10 mM disodium EDTA containing 50 μg of yeast tRNA per ml. Ethanol-precipitated radiolabeled transcripts were analyzed in sequencing gels.

DNA binding assays. Binding of CysB protein to *cysP* promoter DNA was studied by the gel mobility shift method (10, 11) as described previously (34, 40). Binding was carried out in 20 μl of 40 mM Tris-hydrochloride (pH 8.0)–10 mM $MgCl_2$–0.1 M KCl–1 mM dithiothreitol containing 100 μg of bovine serum albumin per ml and 5′-labeled DNA fragments at 0.05 to 0.1 μg/ml. Sonicated calf thymus DNA was included at 1 to 2 μg/ml to reduce nonspecific binding. Mixtures were incubated with purified CysB protein and either *O*-acetyl-L-serine or *N*-acetyl-L-serine for 4 to 8 min at 37°C (incubation times of 0.5 to 15 min give identical results [34]). A 2-μl volume of 50% glycerol containing 0.1% bromophenol blue was then added, and mixtures were immediately loaded onto a 5% polyacrylamide (acrylamide/bis-acrylamide, 82:1) horizontal gel prepared in 10 mM Tris-hydrochloride (pH 8.0)–1 mM disodium EDTA that had been prerun for 30 min at 3 V/cm. After electrophoresis for 60 to 80 min at 10 V/cm, the gel was dried onto filter paper, and radiolabeled bands were visualized by radioautography.

DNase I protection assay. Incubation mixtures were similar to those used for DNA binding studies and contained approximately 10 ng of 5′-labeled DNA fragment (1×10^5 to 2×10^5 dpm), various amounts of purified CysB protein, and *N*-acetyl-L-serine in 50 μl of 40 mM Tris-hydrochloride (pH 8.0)–10 mM $MgCl_2$–0.1 M KCl–1 mM dithiothreitol–100 μg of bovine serum albumin per ml (34). After 5 min at 37°C to allow complex formation, 2 μl of sonicated calf thymus DNA

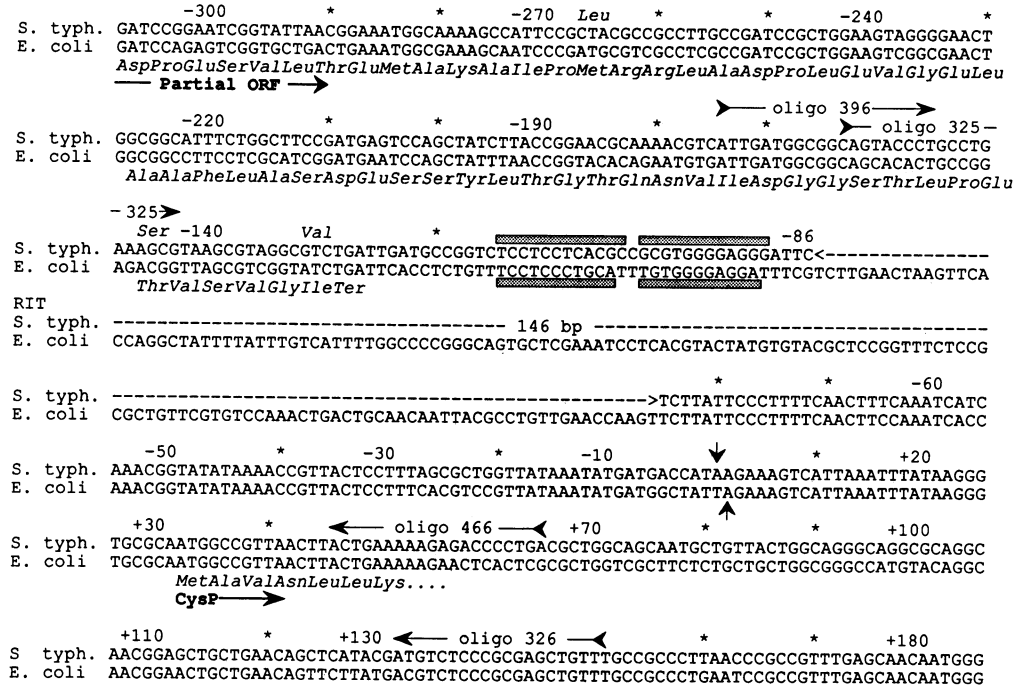

FIG. 1. *cysP* promoter regions of *S. typhimurium* and *E. coli*. The major in vivo transcription start sites are indicated by vertical arrows. Numbering begins at the major *S. typhimurium* start site, which is position +1. The start codon for *cysP* is at position +32. A 146-bp gap was introduced into the *S. typhimurium* sequence to maximize identity with the *E. coli* sequence. Deduced amino acid sequences are shown for the carboxyl end of an unidentified open reading frame at the beginning of the *E. coli* sequence and for the first seven codons of *cysP*. Only differences are shown for *S. typhimurium*. The shaded bars show inverted repeats, which may represent rho-independent terminators (46). The oligodeoxynucleotides used in these studies are indicated with arrows that point from the 5′ and to the 3′ end and correspond to the appropriate strand of the *S. typhimurium* sequence.

(1 mg/ml) and 5 μl of 0.1 M $CaCl_2$ were added, and digestion was initiated with 2 μl of DNase I (0.1 μg/ml). The mixture was incubated at 37°C for an additional 4 min and stopped by the addition of 3.5 μl of 0.25 M disodium EDTA. After extraction with phenol-chloroform-isoamyl alcohol (25:25:1) and the addition of 2 μg of yeast tRNA, DNA was ethanol precipitated, dissolved in water, and analyzed on a sequencing gel.

Other methods. *S. typhimurium* CysB protein, purified through methyl agarose as described previously (29), was estimated to be 85 to 90% pure. *O*-Acetyl-L-serine (47) and *N*-acetyl-L-serine (37) were synthesized as described previously.

RESULTS

DNA sequence of the *S. typhimurium cysP* promoter region. The DNA sequence of the *S. typhimurium cysP* coding region and the 117 bp preceding the ATG start codon has been reported previously (13). An additional upstream sequence was obtained from pMHK1, which is a pT7T318U derivative containing the *cysP* promoter and part of the *cysP* coding region from *S. typhimurium* on a 1.07-kb *Bam*HI-*Eco*RI fragment. A total of 547 bp was sequenced on both strands beginning at the *Bam*HI site, which was found to lie 341 bp upstream of the *cysP* start codon (Fig. 1). This sequence differs at a single position from that previously reported (13), which had an extra A at position −2 relative to the major in vivo transcription start site (see below).

As noted earlier (13), there is a high degree of identity between the *cysP* coding regions of *S. typhimurium* and *E. coli*. In addition, the 116 bp preceding the start codons, which contain the *cysP* promoter (see below), differ at only 10 positions. Insertion of a 146-bp gap in the *S. typhimurium* sequence 117 bp upstream of the coding region gives an alignment in which the identity is extended to the very beginning of our sequence (Fig. 1). This upstream region begins with the carboxyl-terminal portion of a 60-codon open reading frame in which the DNA sequence identity is 80% and the deduced amino acid sequence identity is 95%. The partial open reading frame and the *cysP* open reading frame are separated by 157 bp in the *S. typhimurium* sequence and by 303 bp in the *E. coli* sequence. Immediately downstream of the partial open reading frame, both sequences contain G+C-rich inverted repeats followed by T-rich regions, which are characteristic of rho-independent terminators (46).

In vivo transcription start sites. Primer extension analyses utilized a 5′-labeled oligodeoxynucleotide that was identical to the *S. typhimurium* transcribed strand at 100 to 119 bp downstream of the *cysP* start codon (oligodeoxynucleotide 326; Fig. 1). Extension of this primer with reverse transcriptase and template RNA from wild-type *S. typhimurium* that had been sulfur limited by growth on L-djenkolate gave products of 151, 137, 136, and 101 nucleotides (Fig. 2, lane 3). Larger quantities of these same products were obtained with RNA from sulfur-limited *S. typhimurium*(pRSM16). With growth on L-cystine, no extension products were obtained with identical amounts of template RNA from wild-type cells, and only trace amounts of products were produced with RNA from the pRSM16 strain (Fig. 2, lanes 2 and 4), indicating that *cysP* transcription is under the control of the cysteine regulon. RNA from the sulfur-limited *cysA20*

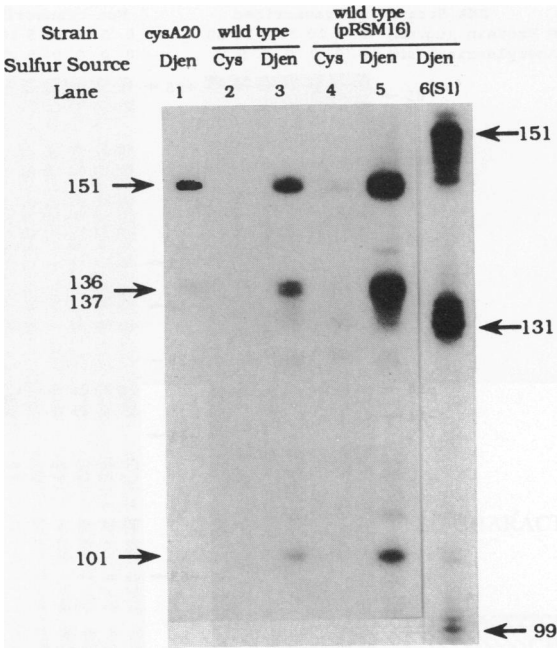

FIG. 2. Identification of in vivo transcription start sites for *S. typhimurium cysP* by primer extension and S1 nuclease protection. DNA fragment lengths are indicated at the left margin for lanes 1 to 5 and at the right margin for lane 6. Oligodeoxynucleotide 326 (Fig. 1) was 5′ labeled with ^{32}P and extended with reverse transcriptase and identical amounts of template RNA from either *cysA20*, the wild-type LT2 strain, or the wild-type LT2 strain carrying pRSM16, which were grown on minimal medium containing either L-cystine (Cys) or the limiting sulfur source L-djenkolate (Djen). Extension products were analyzed on a DNA sequencing gel (lanes 1 to 5) with size standards (not shown) generated by the dideoxy method (49) with the 5′-labeled primer and a DNA template derived from pRSM16. For S1 nuclease protection studies, 50 μg of RNA from sulfur-limited *S. typhimurium* LT2(pRSM16) was hybridized with 50 ng of a 314-bp PCR-generated *cysP* promoter fragment ending 119 bp downstream of the *cysP* start codon (oligodeoxynucleotides 325 and 326; Fig. 1), which was 5′ labeled on the transcribed strand. After treatment with 170 U of S1 nuclease for 20 min at 37°C in a 0.4 ml volume, digests were analyzed on DNA sequencing gels with size standards like those used for primer extension studies.

strain also gave appreciable amounts of these products (Fig. 2, lane 1), which is consistent with our finding that the upstream end of the 4-kb deletion in this strain is just downstream of the *Eco*RI site located 0.74 kb downstream of the *cysP* start codon (14). In the sulfur-limited *cysA20* strain and the wild-type strain the 151-nucleotide product predominated, whereas in wild-type *S. typhimurium*(pRSM16) almost equal amounts of the 151-nucleotide product and the 137- and 136-nucleotide products were obtained.

S1 nuclease protection experiments were performed with a 314-bp PCR-generated *cysP* promoter fragment ending 119 bp downstream of the *cysP* start codon; this fragment was 5′ labeled on the transcribed strand (oligodeoxynucleotides 325 and 326; Fig. 1) and annealed to RNA isolated from sulfur-limited *S. typhimurium*(pRSM16). S1 nuclease digestion gave major DNA products of 151 and 133 to 130 nucleotides as well as small amounts of products of 107, 106, and 98 to 102 nucleotides (Fig. 2, lane 6).

Taken together, these data indicate that the major in vivo transcription start site for *S. typhimurium cysP* is the A located 31 nucleotides upstream of the start codon (Fig. 1). The −10 region corresponding to this start site is TATGAT; the −35 region would lie somewhere within the sequence TTACTCCTT. The smaller primer extension and S1 nuclease digestion products observed in these experiments may have resulted from the degradation of the major *cysP* transcript or may represent activity of other start site(s) downstream of the major start site, e.g., somewhere between positions +15 and +21 relative to the major start site. Start sites farther downstream than position +21 seem unlikely because they would lie beyond the ribosome binding site for *cysP* (13).

A previous study indicated that the major in vivo transcription start site for *E. coli cysP* is situated at the G located 29 bp upstream of the start codon (13). Extension of RNA template from sulfur-limited *E. coli* EC1250 with the same primer used for our *S. typhimurium* studies gave a major product of 150 nucleotides and, after long exposure of the gel, trace products of 136 and 137 nucleotides (data not shown). The longer product corresponds to a major start site at the A located 1 bp upstream of the previously determined position (Fig. 1). This result may be more reliable than that previously reported because we used DNA standards generated by the dideoxy method with the same 5′-labeled oligodeoxynucleotide sequencing primer used for primer extension. The choice in *E. coli* of a start site 1 bp downstream of that used in *S. typhimurium* may be due to the preference of *E. coli* RNA polymerase for initiating RNA synthesis with either an ATP or a GTP (24, 46).

In vitro transcription. *cysP* promoter activity was studied with an in vitro transcription runoff assay utilizing the 314-bp PCR-generated DNA template extending from positions −163 to +151 relative to the major in vivo transcription start site. In the presence of CysB protein and *N*-acetyl-L-serine, RNA runoff products of 153, 151, and 147 nucleotides were formed, corresponding to in vitro transcription start sites at −2, +1, and +5, respectively, relative to the major in vivo start site (Fig. 3). In some experiments small amounts of 152- and 154-nucleotide products were also noted. We observed no runoff products corresponding to initiation at the minor start sites suggested by our in vivo studies.

Very small amounts of runoff products were noted in some experiments with CysB protein alone but were insignificant compared with those obtained when *N*-acetyl-L-serine was added. At the saturating concentration of 5 mM *N*-acetyl-L-serine, large amounts of runoff products were noted with 0.5 μg of CysB protein per ml. A maximal effect was obtained at 1.5 to 2 μg/ml (Fig. 3A). In some experiments (data not shown), significant effects were observed with as little as 0.1 μg of CysB protein per ml. With 2 μg of CysB protein per ml, runoff product formation was markedly stimulated with as little as 0.1 mM *N*-acetyl-L-serine and was half-maximal with *N*-acetyl-L-serine concentrations between 0.2 and 0.4 mM (Fig. 3B). *O*-Acetyl-L-serine was almost as effective as *N*-acetyl-L-serine (data not shown). Sulfide has been shown to inhibit competitively the effects of *N*-acetyl-L-serine on in vitro transcription initiation from the *cysJIH* promoter and is considered an anti-inducer of the cysteine operon (39). A similar effect was observed with the *cysP* promoter, in which 5 mM sulfide increased the amount of *N*-acetyl-L-serine required for a given effect by about fourfold (Fig. 3B).

Effects of thiosulfate on in vitro transcription. Identification of the *cysP* gene product as a periplasmic thiosulfate binding protein (13) prompted us to determine whether thiosulfate affects transcription initiation from the *cysP* promoter. With 1 μg of CysB protein per ml and 0.25 mM *N*-acetyl-L-serine,

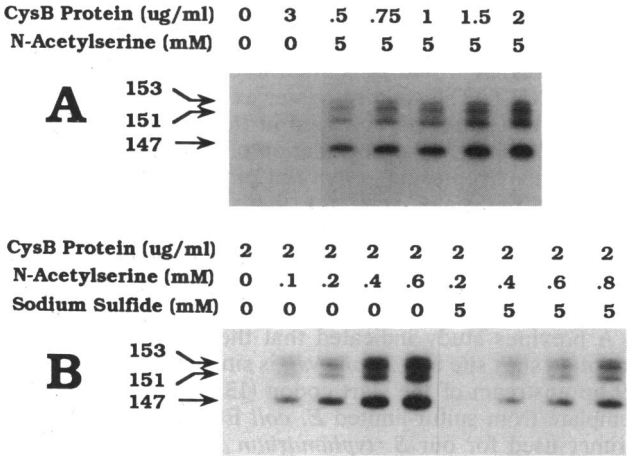

FIG. 3. Effects of CysB protein, *N*-acetyl-L-serine, and sodium sulfide on transcription initiation from the *cysB* promoter in an in vitro runoff assay. Reactions contained 50 μg of RNA polymerase per ml and 1 μg of a PCR-generated 314-bp DNA template extending from positions −163 to +151 relative to the major in vivo transcription start site per ml. Mixtures were preincubated for 5 min at 37°C with various amounts of CysB protein, *N*-acetyl-L-serine, and sodium sulfide, and then radiolabeled transcription runoff products were generated and analyzed on a sequencing gel. Size standards (not shown) consisted of dideoxy sequencing reactions generated from a *cysP* promoter region template and 5′-P^{32}-labeled oligodeoxynucleotide 326 (Fig. 1) as a primer. RNA products of 153, 151, and 147 nucleotides are indicated. (A) CysB protein was varied at a fixed *N*-acetyl-L-serine concentration of 2.5 mM. (B) CysB protein was fixed at 2 μg/ml and *N*-acetyl-L-serine was varied with and without 5 mM sodium sulfide.

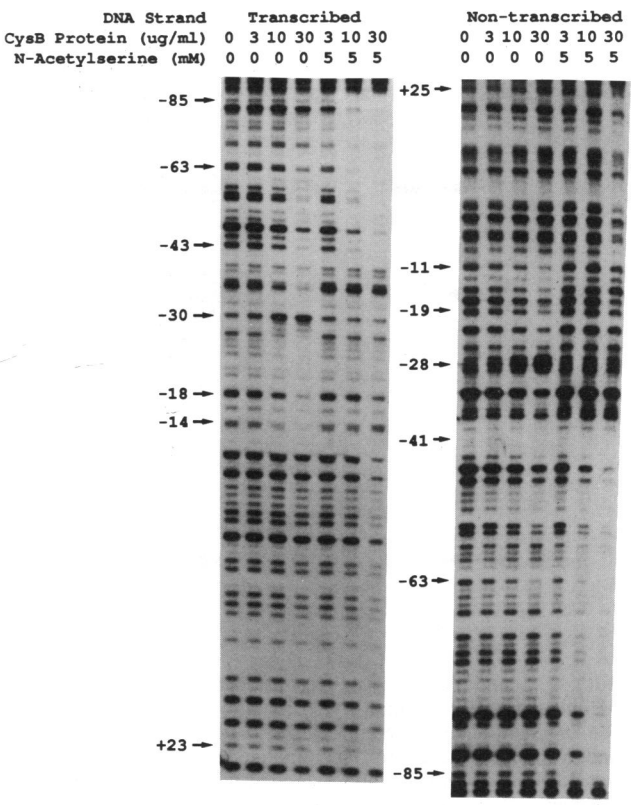

FIG. 5. DNase I protection of the *cysP* promoter region by CysB protein and *N*-acetyl-L-serine. Substrates were generated with the PCR and consisted of a 239-bp fragment extending from positions −174 to +65 relative to the major transcription start site, which was labeled on the transcribed strand, and a 325-bp fragment extending from positions −174 to +151, which was labeled on the nontranscribed strand. CysB protein and *N*-acetyl-L-serine were present as indicated. After digestion with DNase I, the products were analyzed on a DNA sequencing gel. The numbers in the margins refer to positions relative to the major transcription start site.

runoff product formation was inhibited by approximately 70 to 80% with 0.05 mM thiosulfate (Fig. 4). When *N*-acetyl-L-serine was increased to 0.5 and 1 mM, a similar degree of inhibition required 0.1 and 0.25 mM thiosulfate, respectively. Runoff product formation was unaffected by 0.03 to 3 mM sulfate or sulfite or by 5 mM L-cysteine (data not shown). Thiosulfate at 0.05 to 0.25 mM also inhibited transcription initiation with DNA templates containing either the *S. typhimurium cysJIH* or *cysK* promoters but did not affect transcription from a DNA fragment containing the phage λ p_L promoter, even at a concentration of 1 mM, either with or without CysB protein and *N*-acetyl-L-serine (data not shown). The apparent specificity of thiosulfate for *cys* promoters and the reversal of its effects by *N*-acetyl-L-serine establish it as a cysteine regulon anti-inducer that is approximately 100-fold more potent than sulfide.

DNase I footprinting. Protection against DNase I digestion was studied with two different PCR-generated *cysP* promoter fragments, one extending from positions −174 to +65 (oligodeoxynucleotides 396 and 466, Fig. 1) and 5′ labeled on the transcribed strand and the other extending from positions −174 to +151 (oligodeoxynucleotides 396 and 326, Fig. 1) and 5′ labeled on the nontranscribed strand. CysB protein alone at 10 and 30 μg/ml partially protected the transcribed strand from positions −63 to −14 and created a hypersensitive site at position −30 (Fig. 5). The region of protection on the nontranscribed strand extended from positions −63 to −11, and hypersensitive sites were noted at positions −29, −28, and −27 (Fig. 5).

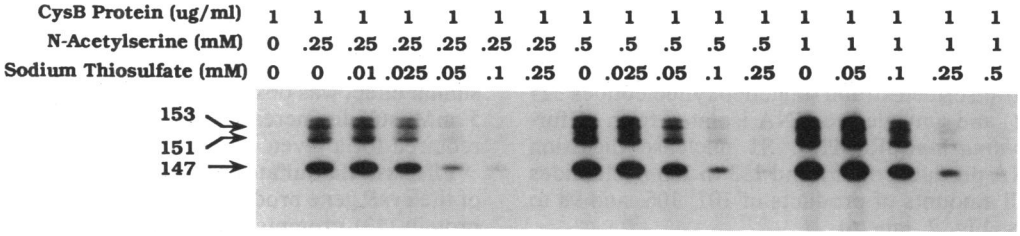

FIG. 4. Inhibition of in vitro transcription initiation by thiosulfate. Transcription runoff assays were carried out as described in the legend to Fig. 3 with various amounts of *N*-acetyl-L-serine and sodium thiosulfate. RNA products of 153, 151, and 147 nucleotides are indicated.

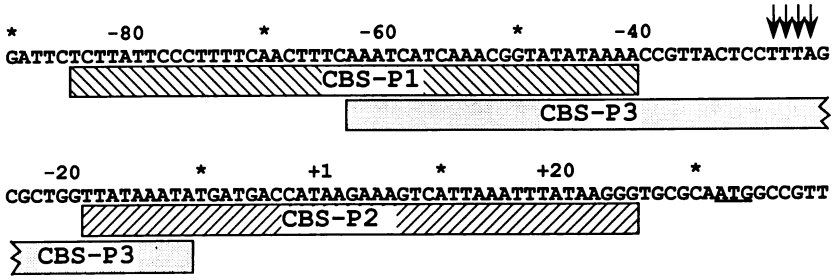

FIG. 6. Binding sites for CysB protein in the *S. typhimurium cysP* promoter region. Protection against DNase I digestion was noted at CBS-P3 in the absence of *N*-acetyl-L-serine and at CBS-P1 and CBS-P2 in the presence of *N*-acetyl-L-serine. The vertical arrows represent sites that are made hypersensitive to DNase I digestion by CysB protein in the absence of *N*-acetyl-L-serine.

The addition of 5 mM *N*-acetyl-L-serine markedly enhanced the effects of CysB protein but also shifted the boundaries of the protected area to positions −85 to −43 on the transcribed strand and −85 to −41 on the nontranscribed strand (Fig. 5). In addition, the hypersensitive sites were lost and a new downstream area of protection was observed at positions −18 to +23 on the transcribed strand and −19 to +25 on the nontranscribed strand. Protection in this downstream region was less pronounced than that observed upstream and required higher concentrations of CysB protein. Using the convention adopted for the CysB protein binding sites previously described for the *cysK*, *cysJIH*, and *cysB* promoters (34, 41), we designated the region between positions −85 and −41 as CBS-P1 and the region between positions −19 and +25 as CBS-P2. The region between positions −63 and −11, which is protected in the absence of *N*-acetyl-L-serine, is designated CBS-P3 (Fig. 6).

Gel mobility shift binding studies. Preincubation of CysB protein with the 5′-labeled, 325-bp PCR-generated fragment extending from positions −174 to +151 gave a DNA complex with an electrophoretic mobility of 0.62 relative to that of uncomplexed DNA (Fig. 7, lanes 2 to 4). With 0.2 μg of CysB protein per ml, this complex, which we refer to as the slow complex, contained about 70% of the total input DNA. *O*-Acetyl-L-serine had a variable effect on binding affinity, slightly increasing it in some experiments and slightly decreasing it in others, but in all cases it increased complex mobility in a concentration-dependent manner with a half-maximal effect at about 1 mM. Saturating concentrations of *O*-acetyl-L-serine gave a complex, which we refer to as the fast complex, with a mobility of 0.73 relative to that of uncomplexed DNA (Fig. 7, lanes 5 to 10). Fast complex formation and mobility were unaffected by *N*-acetyl-L-serine, sulfide, and thiosulfate at 10, 30, and 3 mM, respectively (data not shown).

A similar pattern of slow and fast complexes has been described for interactions between CysB protein and *cysK* promoter fragments, where it is thought that the slow complex contains bent DNA, which is known to migrate more slowly during gel electrophoresis (53), and that the fast complex contains DNA that is bent less or not at all (34). We have postulated that *O*-acetyl-L-serine prevents DNA bending by CysB protein, and that the electrophoretic bands with intermediate mobilities observed with nonsaturating concentrations of *O*-acetyl-L-serine represent mixtures of bent and unbent DNA complexes that are in rapid equilibrium during electrophoresis (34). We also believe that the failure of *N*-acetyl-L-serine, sulfide, and thiosulfate to affect complex migration is due to migration of these anions away from DNA-CysB protein complexes during electrophoresis (34). The reactivity of the neutral compound *O*-acetyl-L-serine in the gel mobility shift assay is thought to result from its gradual conversion at the top of the gel to *N*-acetyl-L-serine, which then migrates toward and overtakes the complexes (34).

Bending of the *cysK* promoter DNA by CysB protein appears to occur at or near a point between two adjacent CysB protein binding sites, CBS-K1 and CBS-K2 (34). CysB protein creates hypersensitive sites in this region; these sites disappear in the presence of *O*-acetyl-L-serine. Since the effects of DNA bending on electrophoretic mobility are known to be position dependent and greater when bending occurs closer to the middle of the fragment (53), we compared the behavior of two 5′-labeled *cysP* promoter fragments in which the DNase I-hypersensitive sites at positions −30 to −27, approximately midway between CBS-P1 and CBS-P2, were situated either near the middle or close to one end. The 325-bp PCR-generated fragment extending from positions −174 to +151, which was characterized above, contains these sites at 45% of the distance from one end. We

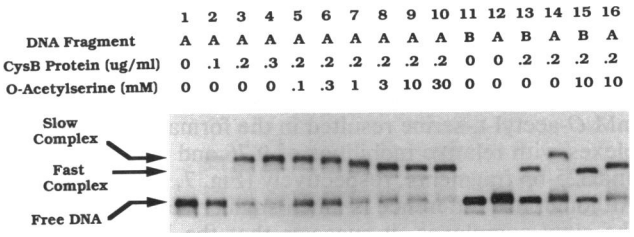

FIG. 7. Binding of CysB protein to a *cysP* promoter fragment in gel mobility shift assays (11, 12). Approximately 0.1 μg of 5′-labeled *cysP* promoter fragment per ml was preincubated with various amounts of CysB protein and *O*-acetyl-L-serine for 5 min at 37°C. Sonicated calf thymus DNA was also present at 1.5 μg/ml to reduce nonspecific binding. Mixtures were then run in a 5% polyacrylamide gel as described in Materials and Methods. With a 325-bp fragment extending from positions −174 to +151 relative to the major transcription start site (fragment A), the CysB protein in the absence of *O*-acetyl-L-serine gave a band designated the slow complex (lanes 2 to 4). *O*-Acetyl-L-serine increased the mobility of complexed DNA in a concentration-dependent manner (lanes 5 to 10). The most rapidly migrating complex is designated the fast complex. Lanes 11 to 16 compare the effects of CysB protein with DNA fragments containing DNase I hypersensitivity sites positioned either centrally or at one end. These sites (positions −30 to −27) were located at 45% of the length of the 325-bp fragment A and at 19% of the length of the 320-bp fragment B, which extended from positions −89 to +231. Both fragments gave slow and fast complexes in the absence and presence of *O*-acetyl-L-serine, respectively.

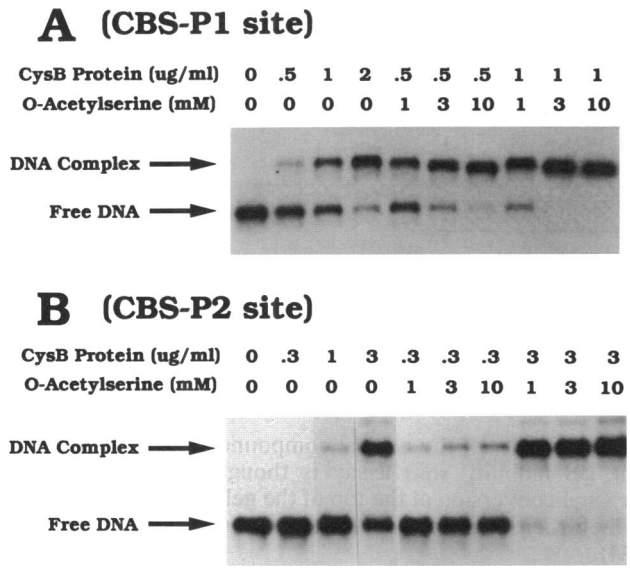

FIG. 8. Binding of CysB protein to *cysP* promoter fragments containing either CBS-P1 or CBS-P2 alone. The conditions of the gel mobility shift assay were the same as those described in the legend to Fig. 7. Concentrations of CysB protein and *O*-acetyl-L-serine are indicated. (A) Reactions contained a 0.25-μg/ml quantity of a 5′-labeled CBS-P1 fragment including the *cysP* promoter sequence between positions −174 and −27 and 100 bp of sequence from pT7T319U. (B) Reactions contained a 0.1-μg/ml quantity of a 5′-labeled 87-bp CBS-P2 fragment extending from positions −22 to +65.

also generated a 5′-labeled 320-bp *Hin*fI-*Hpa*I restriction fragment, which extends from positions −89 to +231 and contains these sites at 19% of the distance from one end. The relative mobilities of the slow complexes formed in the absence of *O*-acetyl-L-serine were 0.73 for the 320-bp fragment and 0.62 for the 325-bp fragment (Fig. 7, lanes 13 and 14). These differences are too large to be accounted for by the slightly larger size of the 325-bp fragment and suggest that the slow complex contains DNA that is bent at or near the region between CBS-P1 and CBS-P2. The addition of 10 mM *O*-acetyl-L-serine resulted in the formation of fast complexes with relative mobilities of 0.76 and 0.73 for the 320- and 325-bp fragments, respectively (Fig. 7, lanes 15 and 16). Although this difference is much smaller than that found for the slow complexes, it suggests that the DNA in the fast complexes may be slightly bent. Studies of the *cysK* promoter are also consistent with a small amount of bending in the fast complex (34).

Interactions of CysB protein with individual binding sites. DNA containing CBS-P1 alone was prepared by PCR from a pMHK7 template with oligodeoxynucleotide 396 (Fig. 1), which extends from position −174 toward CBS-P1, and a 5′-labeled 26-mer (5′-CCAGGGTTTTCCCAGTCACGACGT TG-3′) complementary to the pT7T319U vector sequence beginning 81 bp from and extending toward the polylinker region. This 248-bp fragment contained positions −174 to −27 of the *cysP* promoter followed by 100 bp of vector sequence. In the gel mobility shift assay (Fig. 8A), half-maximal binding to the CBS-P1 fragment in the absence of *O*-acetyl-L-serine occurred with about 1 μg of CysB protein per ml, which is approximately five times more than the amount required for an equivalent effect with the fragment containing both binding sites (Fig. 7). *O*-Acetyl-L-serine stimulated complex formation approximately twofold and also increased the relative mobility of the complex from 0.67 to 0.72, indicating that the complex formed with CBS-P1 alone in the absence of *O*-acetyl-L-serine may also be bent. Position −28 is located about 41% of the distance from one end of this fragment.

For studies with CBS-P2, a 5′-labeled PCR-generated fragment extending from positions −174 to +65 was digested with *Hae*II to give a 97-bp radiolabeled product, extending from positions −22 to +65, which was purified by polyacrylamide gel electrophoresis. CysB protein bound to this fragment with a half-maximal effect noted at slightly less than 3 μg of CysB per ml in the absence of *O*-acetyl-L-serine (Fig. 8B). *O*-Acetyl-L-serine stimulated binding to the CBS-P2 fragment but did not increase the mobility of complexed DNA.

DISCUSSION

The data presented here identify a major transcription start site for the *cysP* promoter located 31 bp upstream of the *cysP* start codon and demonstrate a requirement for CysB protein and the inducer acetyl-L-serine for in vitro transcription initiation. In this communication, we use the term acetyl-L-serine to mean *O*-acetyl-L-serine and/or *N*-acetyl-L-serine. The differences between the two have been discussed previously (34, 39) and are mostly operational. For instance, both are active in transcription runoff experiments and DNase I protection studies and in gel mobility shift studies with DNA fragments containing a single binding site, e.g., the *cysJ* promoter and the CBS-K2 site of the *cysK* promoter. Only *O*-acetyl-L-serine is active, however, in gel mobility shift studies with the intact *cysK* and *cysP* promoters, in which the two isomers are added to the binding assay but are not included in the electrophoresis buffer. We think this is due to the ability of *O*-acetyl-L-serine to provide a continuous source of *N*-acetyl-L-serine to the gel through its isomerization to the latter, which then migrates anodally in the direction of the migrating complex. With the *cysK* promoter, *N*-acetyl-L-serine has been shown to be active when added to the electrophoresis buffer (34). A similar study has not been performed with the *cysP* promoter.

Our transcription runoff experiments also indicate that thiosulfate is more potent than sulfide as an anti-inducer of the *cysP*, *cysJIH*, and *cysK* promoters and indirectly support the notion that thiosulfate is a physiologically important end product of inorganic sulfur reduction. Nakamura et al. (35, 36) have shown in *S. typhimurium* that *O*-acetylserine (thiol)-lyase B, encoded by *cysM*, catalyzes the synthesis of *S*-sulfocysteine from *O*-acetyl-L-serine and thiosulfate. Presumably, *S*-sulfocysteine is then hydrolyzed to L-cysteine and sulfate or reduced by glutathione to L-cysteine and sulfite (52), thereby eliminating the need to reduce inorganic sulfur to the level of sulfide. *S. typhimurium cysM* strains are Cys^+ under aerobic conditions owing to the presence of *O*-acetylserine (thiol)-lyase A, which is encoded by *cysK* and uses sulfide as a substrate for L-cystine biosynthesis (4, 23). When grown anaerobically, however, *cysM* mutants are cysteine bradytrophs, suggesting that in its natural enteric environment, which is essentially anaerobic, *S. typhimurium* prefers to synthesize L-cysteine from thiosulfate via *S*-sulfocysteine.

The *cysP* promoter lacks a consensus −35 region, a condition that is characteristic of regulation by an activator protein (12, 45), and is found in the *cysJIH* and *cysK* promoters (34, 39). Although all three promoters are positively regulated by CysB protein and acetyl-L-serine, they

TABLE 1. Properties of CysB protein binding sites in *S. typhimurium cys* promoters

Promoter[a]	Regulation by CysB protein	Binding site	Position relative to transcription start site	Proposed function	Effect of acetyl-L-serine[b] on:	
					Binding avidity[c]	Complex mobility[c]
cysJIH	Positive	CBS-J	−76 to −35	Transcription activation	Increase	None
cysP	Positive	CBS-P1	−85 to −41	Transcription activation	Increase	Increase
		CBS-P2	−19 to +25	Unknown	Increase	None
		CBS-P3	−63 to −11	Unknown		Large increase
cysK	Positive	CBS-K1	−78 to −39	Transcription activation	Increase	None
		CBS-K2	−115 to −79	Unknown	Decrease	None
cysB	Negative	CBS-B	−10 to +36	Negative autoregulation	Decrease	None

[a] The data for the *cysJIH* and *cysK* promoters are from Monroe et al. (34); the data for the *cysB* promoter are from Ostrowski and Kredich (41).
[b] Either *O*-acetyl-L-serine or *N*-acetyl-L-serine.
[c] Measured in a gel mobility shift assay.

differ significantly with respect to the number, arrangement, and reactivity of binding sites for CysB protein (Table 1). The *cysJIH* promoter is the simplest with a single site, CBS-J, located immediately upstream of the −35 region (34, 39). The *cysK* promoter (34) and the *cysP* promoter described here also contain binding sites immediately upstream of the −35 region; these binding sites are designated CBS-K1 and CBS-P1. CBS-K1 has been demonstrated by mutational analyses to be required for in vivo activity of the *cysK* promoter (34); because the positions of *cysK* and *cysP* relative to the −35 regions of their promoters are similar, we assume that CBS-J and CBS-P1 serve the same function.

The *cysB* promoter also has a single CysB protein binding site, designated CBS-B, which is located between positions −10 and +36 relative to the major transcription start site. Binding of the CysB protein to CBS-B inhibits in vitro transcription initiation (41) and is probably responsible for negative autoregulation of *cysB* (5, 17). CBS-B differs from the three sites associated with positive regulation with respect to the effects of acetyl-L-serine, which inhibits binding of CysB protein and also partially reverses the inhibition of in vitro transcription initiation. This property may allow a fine tuning of autoregulation in response to sulfur availability and inducer levels (41).

In contrast to the *cysJIH* and *cysB* promoters, the *cysP* and *cysK* promoters have second discrete binding sites for CysB protein, which differ in position and in their reactivity with acetyl-L-serine in gel mobility shift experiments. As shown here, CBS-P2 is downstream of CBS-P1 and separated from it by 21 bp, whereas CBS-K2 is upstream of and contiguous with CBS-K1 (Table 1). Acetyl-L-serine stimulates binding of CysB protein to CBS-P2 but inhibits binding to CBS-K2. Deletion of CBS-K2 does not alter *cysK* promoter strength in vivo, and at present its function is unknown. We have speculated that this site may help sequester CysB protein at the *cysK* promoter when sulfur is replete, thereby ensuring a rapid increase in *cysK* expression in the event of a sudden decrease in sulfur availability (34). The same could hold true for CBS-P2. An alternative possibility is that binding of CysB protein to CBS-P2 represses transcription by interfering with RNA polymerase binding. To test this possibility, we investigated the effects of high CysB protein concentrations in a runoff assay and found that with 3 mM *N*-acetyl-L-serine transcription initiation was maximally stimulated by CysB protein at 3 μg/ml but was inhibited by approximately 60 and 90% with CysB protein at 20 and 40 μg/ml, respectively (data not shown). In contrast, transcription initiation from the phage λ p_L promoter was unaffected by CysB protein at concentrations as high as 50 μg/ml, with or without *N*-acetyl-L-serine. The significance of these findings is unclear, however, since these high concentrations of CysB protein were about half as effective in inhibiting transcription initiation from the *cysJIH* promoter, which does not have a downstream binding site (data not shown).

DNA sequence comparison of the *S. typhimurium* CBS-P1, CBS-K1, and CBS-J binding sites and the corresponding regions in *E. coli* gives a consensus sequence with 12 identical nucleotides for all six sequences over a distance of 40 bp (Fig. 9). It should be noted that the *cysP* promoter sequences must be offset by 12 to 14 bp relative to the transcription start site for maximum identity with the *cysK* and *cysJIH* promoters. Although CysB protein binding sites have not been demonstrated for the *E. coli cysD* promoter, the sequence immediately upstream of the −35 region of that promoter (25) can also be aligned to show nucleotide identities at 8 of these 12 positions (data not shown). As noted previously (34, 41), CBS-K2 and CBS-B resemble each other more than they do either CBS-K1, CBS-J, or CBS-P1 (Fig. 9). This may be related to the fact that acetyl-L-serine inhibits binding of CysB protein to the former and stimulates binding to the latter. This correlation holds true for the CBS-P2 sequence, which is similar to that of CBS-P1 and also binds CysB protein more tightly in the presence of acetyl-L-serine. The inverted CBS-P2 sequence is identical to CBS-P1 at 25 of 44 positions and to the consensus sequence found for CBS-K1, CBS-J, and CBS-P1 at 9 of 12 positions. CBS-P2 itself contains an inverted repeat with identical nucleotides at 15 of 19 positions centered around position +2 (Fig. 9). As a result, the noninverted sequence also resembles CBS-P1 with identical nucleotides at 20 of 45 positions and is identical to the consensus sequence at 7 of 12 positions (data not shown).

The results of our DNase I protection experiments with the *cysP* promoter are more complex than those obtained with the *cysJIH*, *cysK*, and *cysB* promoters (34, 41) and require some speculation to be reconciled with the results of our gel mobility shift binding assays. We propose that binding of CysB protein to the CBS-P3 site in the absence of acetyl-L-serine causes the DNA to bend at a point approximately midway between CBS-P1 and CBS-P2, resulting in anomalously slow electrophoretic mobility and DNase I hypersensitivity at positions −30 to −27. We cannot yet say whether this complex contains one or two CysB protein molecules, but in either case binding to CBS-P3 inhibits binding to the overlapping CBS-P1 and CBS-P2 sites. Acetyl-L-serine changes the conformation of CysB protein in such a way that it binds preferentially to CBS-P1 rather than

Binding of CysB Protein Stimulated by O-Acetylserine

```
St  CBS-K1  (-78)  ATAACCATTATTTCCCATCAGCATATAGATATGCGAAATCCTTACTTCCCCAT (-26)
Ec "CBS-K1" (-80)  CATGTCATTATTTCCCTTCTGTATATAGATATGCTAAATCCTTACTTCCGCAT (-28)
St  CBS-J   (-79)  AAACAGGTTAGTTCATTTGGTTATTT#GTTATTTCCAACCCTTCTTTAATTGT (-28)
Ec "CBS-J"  (-78)  AAACAGGTTAGTCGATTTGGTTATTA#GTTATCGCTATCCCGTCTTTAATCCA (-27)
St  CBS-P1  (-90)  GATTCTCTTATTCCCTTTTCAACTTT#CAAATCATCAAACGGTATATAAAACC (-40)
Ec "CBS-P1" (-90)  TTTCGTCTTATTCCCTTTTCAACTTC#CAAATCATCAAACGGTATATAAAACC (-40)
Consensus                TTA.T.....T.....T.. ...AT....A..C..T...T

inv repeat                          
St-P2 (inv)      (+25)  CCCTTATAAATTTAATGACTTT#CTTATGGTCATCATATTTATAACC   (-19)
Identity with CBS-P1    C.CTTAT....TT....ACTTT C..AT..TCA.....T.TATAA
```

Binding of CysB Protein Inhibited by O-Acetylserine

```
St CBS-B    (-10)  TAGTGGTTATAGTTAACACCTTTTTTATTATTAAATCGTATTAGCA (+36)
Ec "CBS-B"  (-10)  TAGTGGTTATAGTTAGCACCTTTTTTATTATTAAATCGTATTAGTC (+36)
St  CBS-K2  (-117)  AAATAAGAGATGGCTTATGCTGTCTCTTATTCCATACTGATA (-76)
Ec "CBS-K2" (-119)  GAAACAGGGGTGGCTTATGCCGCCCCTTATTCCATCTTGCAT (-80)
Consensus                        A.........TTATT..AT..T
```

FIG. 9. Comparison of CysB protein binding sites from *S. typhimurium* (St) and the corresponding regions in *E. coli* (Ec). Sequences are grouped according to whether binding of CysB protein is stimulated or inhibited by *O*-acetyl-L-serine. The *E. coli* "CBS-K1," "CBS-K2," "CBS-P1," and "CBS-B" sequences are from strain K12 (6, 13, 41), and the *E. coli* "CBS-J" sequence is from strain B (39). A single gap (#) was introduced into CBS-J and CBS-P1 to increase the number of nucleotides identical to those of CBS-K1. The inverted CBS-P2 sequence showed the best identity with CBS-P1. CBS-P2 itself contains an inverted repeat, which is shown above the inverted sequence as shaded bars. The *E. coli* "CBS-P2" sequence is not shown but is identical to that of *S. typhimurium* CBS-P2 at 40 of 44 positions (Fig. 1).

to CBS-P3. Binding to CBS-P1 causes little or no bending, and therefore complex mobility is increased and DNase I hypersensitivity is lost.

In this model, the removal of the CysB protein from the CBS-P3 site would also permit binding of the CysB protein to the CBS-P2 site, although with less avidity than to CBS-P1 as evidenced by the lesser degree of DNase I protection found for CBS-P2. Protection of both sites in the presence of acetyl-L-serine almost certainly involves binding by two separate CysB protein molecules, since a single 144-kDa tetramer would have to be unusually elongated to protect regions that are separated by as many as 110 bp. DNA bending would allow a single molecule to protect over a large distance, but our gel mobility shift data indicate that acetyl-L-serine decreases rather than increases DNA bending while it extends the boundaries of DNase I protection. It is not clear, however, whether the fast complex observed in our gel mobility shift assays contains two CysB protein molecules, since these experiments were done at CysB protein concentrations of less than 1 μg/ml and the DNase I protection studies were done with 3 to 30 μg of CysB protein per ml. From gel mobility shift studies, we estimate that the CysB protein concentrations necessary for half-maximal binding in the presence of *O*-acetyl-L-serine are approximately 0.6, 0.1, and 0.1 μg/ml, respectively, for fragments containing either CBS-P2 alone, CBS-P1 alone, or both sites together. With the CysB protein concentration of 0.2 μg/ml used to titrate the effects of *O*-acetyl-L-serine on the mobility of complexes containing both sites (Fig. 7), independent binding to CBS-P2 would have been insufficient to form a two-protein complex unless the affinity for this site were markedly increased through a cooperative interaction with another CysB protein molecule bound to CBS-P1. With CysB protein concentrations of 1 μg/ml and higher, we noted an additional band that migrated more slowly than the slow complexes and may represent a two-protein complex (data not shown).

Binding of CysB protein to the CBS-K1 and CBS-K2 sites of the *cysK* promoter in the absence of acetyl-L-serine also gives a slow complex, which appears to contain bent DNA (34). In this case, however, acetyl-L-serine inhibits binding to CBS-K2, making it likely that the fast complex contains only a single CysB protein molecule bound to CBS-K1. The effects of acetyl-L-serine on complex mobility were found to require DNA fragments containing both CBS-K1 and at least the downstream two-thirds of CBS-K2, suggesting that DNA bending is due to binding to both sites. In contrast, acetyl-L-serine increases the electrophoretic mobility of a CBS-P1 fragment completely lacking CBS-P2. We have not yet determined whether the intersite sequence between positions −40 and −27, which was present in our CBS-P1 fragment, is required for this effect.

To formulate a model that is applicable to both the *cysK* and *cysP* promoters, we propose that the slow complex noted in the gel mobility shift experiments with relatively low CysB protein concentrations in the absence of acetyl-L-serine contains a single CysB protein molecule bound to both sites, which induces DNA bending. Acetyl-L-serine stimulates binding to the sites required for positive regulation, i.e., CBS-K1 and CBS-P1, and also decreases or eliminates DNA bending, giving rise to fast complexes, which also contain only a single CysB protein molecule. With the higher CysB concentrations required for DNase I protection experiments, a second CysB protein molecule can bind specifically to CBS-P2, resulting in a two-protein complex, but this does not occur with the *cysK* promoter, in which binding to CBS-K2 is inhibited by acetyl-L-serine. Careful measurements of the stoichiometry of binding in these complexes will be required to distinguish between this model and others involving binding of multiple CysB protein molecules.

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